University of Alberta

Incorporating Low Relative Humidity and Low Temperatures into Tree Nursery Growing Regimes: Effects on White Spruce Seedling Growth, Morphology and Physiology

by

Jessica Jaye Edna Roberts ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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The undersigned certify that they have read and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis titled "Incorporating Low Relative Humidity and Low Temperatures into Tree Nursery Growing Regimes: Effects on White Spruce Seedling Growth, Morphology and Physiology", submitted by Jessica Jaye Roberts in partial fulfillment of the requirements for the degree of Master of Science. in Forest Biology and Management.

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Abstract

White spruce seedlings were grown from seed and exposed to low, medium or high relative humidity (RH) levels during their early and rapid growth stages. Lower RH levels produced shorter seedlings, with narrower stem diameters and shorter and denser needles. Once flushed and grown under low RH conditions, the seedlings originally grown under low RH flushed sooner, had greater absolute height growth, longer and less dense needles, and maintained higher photosynthetic levels, than those reared under high humidity levels.

In a separate experiment, seedlings were periodically exposed to 5°C for three different frequencies during their early and rapid growth phases. Greater frequency of exposure produced seedlings with shorter shoot heights, but similar stem diameters. No difference in needle length or density were found between treatments. Seedlings frequently exposed to chilling maintained higher shoot water potentials during a severe water stress, and recovered closer to pre-stress levels compared to the control seedlings. Frequent chilling exposure may also increase the frost tolerance of unhardened seedlings.

Dedication

I would like to dedicate this thesis, in part, to Dr. Louis Druehl; one of my mentors and more importantly, a personal friend. Louis, you were one of the first people to believe in me and give me a chance when others didn't, and for that I will always be grateful. You opened my eyes to Science, and helped me appreciate that not all science is conducted as a means to an end. You supported me in my decision to leave marine biology, regardless if it was the right decision or not. Without your encouragement and understanding I would never had started this project. Thank you.

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Chapter I: General Introduction

Successful establishment and growth of outplanted, nursery-grown seedlings depends on numerous factors: seed source, nursery cultural regimes, seedling quality, duration of cold storage, handling during transport and planting, as well as the environmental and physical microsite conditions of the planting site (Duryea 1985; Grossnickle *et al.* 1991a, 1991b; Simpson *et al.* 1994; Jiang *et al.* 1995; Proctor 1996; Kimmons 1997). Of these, seedling stock quality is of high importance. If environmental factors are favorable for outplanting but the seedling stock is of poor quality, low seedling survival and establishment may result. Conversely, if the planting stock is of acceptable quality, but the environmental conditions at the planting site are harsh, then again, the seedlings may have low survival rates (Dunsworth 1997; Kimmons 1997). Therefore, the first steps towards successful reforestation involve identifying the environmental parameters of the forest planting site, then producing seedlings with the morphological and physiological characteristics necessary for survival and growth on that site.

Tree nursery cultural practices significantly influence the quality of seedling planting stocks. Nutrient and water supply, light intensity, photoperiod, day and night temperatures and the humidity level under which seedlings are reared are all factors that may influence their morphological and physiological characteristics (Landis *et al.* 1989a, 1992; Van den Driessche 1991a, 1991b; Wood 1995; Marsden 1995; Folk and Grossnickle 1997; Mattsson 1997). By changing one or more of these factors, tree growers are able to predictably alter seedling morphological characteristics, including seedling root to shoot ratio, shoot height, stem diameter, and needle area (van den Driessche 1991b; Wood 1995; Lamhamedi *et al.* 1997). Physiological characteristics can also be affected, including carbohydrate accumulation and storage, cold hardiness, drought resistance, root growth capacity and stomatal sensitivity (Marshall 1985; DeYoe *et al.* 1988; Zwiazek and Blake 1989; van den Driessche 1991a, b; Edwards and Dixon 1995; Lamhamedi *et al.* 1997). Therefore, by altering the environmental conditions under which seedlings are grown, tree growers can potentially produce seedlings with characteristics that can tolerate outplanting stresses.

Currently, tree nurseries rear white spruce seedlings under relatively stress-free environmental conditions during the early and exponential phases of seedling development (Landis *et al.* 1989a, 1992; Wood 1995). This produces a seedling stock with a general morphology and physiology that is expected to perform well on all outplanting sites. However the low-stress environmental conditions found within a nursery greenhouse often do not reflect those found on an open-canopy forest site. When the tree canopy is removed, increased incidents of wind gusts, morning and night time frost events, higher daytime temperatures and lower humidity levels occur (Jordan and Smith 1995; Balisky and Burton 1995; Marsden *et al.* 1996; Man and Lieffers 1997). It is

thus questionable if producing tree seedlings under the currently recommended nursery conditions is the best method of preparing them for outplanting onto open-canopy forest sites. More specifically, can nursery tree growers prescribe an alternative growing regime that would produce a planting stock that is better adapted to high-stress field conditions?

Humidity is acknowledged as one of the primary factors responsible for regulating the growth of containerized conifer seedlings (Landis *et al.* 1992; Wood 1995). High humidity levels can be detrimental to a seedling crop, as excess water vapor condenses on either the seedlings, or their container walls. This environment promotes the growth and propagation of opportunistic or pathogenic organisms, such as mosses, algae, liverworts, fungi, molds, bacteria or insects (Landis *et al.* 1989b). Conversely, excessively low humidity levels could potentially place water stress on the seedlings. Low environmental relative humidity levels can reflect a high vapor pressure deficit (VPD), which directly affects seedling stomatal behavior, and transpiration rates. High vapor pressure deficits initially induce high transpiration rates in seedlings, causing them to lose water and turgor, and in later stages may result in closure of the stomata and a reduction in the transpiration stream (Sheriff 1977; Nonami *et al.* 1990; Assmann *et al.* 1991). Extended conditions of high VPD could also lead to a decrease in seedling photosynthesis, relative to conditions of low VPD.

To date, few studies have isolated the specific effects of humidity (or humidity-related parameters) on the growth and development of containerized conifer species, since humidity is dependent on temperature. The main focus has been on horticultural species (Krizek *et al.* 1971; Sheriff 1977; Morrison Baird and Webster 1978; Seiler and Johnson 1984; Van de Sanden 1985). However, tree growers have expressed the need for controlled experiments to determine precisely how humidity affects conifer seedling morphology and physiology (Landis *et al.* 1992). Since low humidity levels are commonly found on open-canopy forest sites (Marsden *et al.* 1996; Man and Lieffers 1997), it is necessary to determine exactly how this environmental parameter affects the establishment and subsequent growth of nursery-grown seedlings. It is also important to establish how different humidity levels during seedling production affect their subsequent growth under various environmental conditions.

Nursery greenhouse temperature influences all aspects of seedling growth and development. It is currently recommended that nurseries maintain greenhouse temperatures of 17-22°C during the initial 18 to 20 weeks of white spruce seedling production (Landis *et al.* 1992; Wood 1995). However, maintaining these temperatures during the winter months, when some northern tree nurseries seed their containers (Draper and Hawkins 1989), can lead to high greenhouse heating costs. Investigating alternative growing regimes that incorporate low greenhouse temperatures, could lead to reduced heating costs for nurseries (Landis *et al.* 1992). However, the effects of low temperature on the growth, development and general stress resistance of the seedlings should be considered before implementing changes to current growth regimes. Previous studies indicate that preconditioning plants with low levels of stress increases their

subsequent tolerance to that stress (De Yoe *et al.* 1988; van den Driessche 1991a, 1991b; Anderson *et al.* 1994, 1995; Zhao *et al.* 1995). Increased frost tolerance would be potentially beneficial for seedlings spring planted onto open-canopy harvested sites, where loss of the protective tree canopy may result in increased incidents of periodic frost episodes. Therefore, incorporating low temperatures into nursery growing practices may have beneficial biological and economic implications for northern tree nurseries.

The purpose of this research was to investigate alternative growing regimes for tree nurseries. The main objective was to determine how different humidity levels and periodic low temperature exposure, during initial white spruce [*Picea glauca* (Monech) Voss] seedling development affects their morphological and physiological characteristics. Humidity and low temperature effects were studied independently in two separate, concurrent experiments. The specific objectives of the two studies were as follows:

Objectives: Humidity Experiment

1. To determine the effects of relative humidity (during the early and rapid growth stages of white spruce seedling growth) on needle morphology and epicuticular wax production.

2. To determine if the initial humidity effects on morphology are persistent after subsequently flushing and growing the seedlings under high or low humidity levels.

3. To determine if initial nursery growing regimes influence the frost tolerance and drought resistance of unhardened seedlings.

4. To determine if the initial nursery humidity levels influence the subsequent timing of seedling bud flush.

5. To determine if initial nursery growing regimes affect gas exchange parameters under subsequent conditions of high and low relative humidity.

Objectives: Periodic Low Temperature Experiment

1. To determine if periodically exposing white spruce seedlings to 5°C affects seedling growth or morphology, compared to control seedlings.

2. To determine if periodic chilling exposure of seedlings increases the frost tolerance or drought resistance of unhardened seedlings.

3. To determine if periodic chilling affects the root growth capacity of the seedlings following cold storage.

4. To determine if periodic chilling influences seedling survival, growth or morphological characteristics after outplanting for one season.

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Chapter II: Effects of Relative Humidity on White Spruce (*Picea glauca*) Physiology

Introduction

Containerized white spruce [*Picea glauca* (Monech) Voss] seedlings undergo six principle developmental stages before they are outplanted: germination, early growth, rapid growth, bud initiation, stem finishing and, if they are to be cold stored, cold conditioning (Landis *et al.* 1992; Wood 1995). Each of these growth stages have different requirements for water and nutrient supply, photoperiod, temperature and humidity. Traditionally, moderately-high to high humidity levels are maintained during the germination, early and rapid growth phases to promote a stress-free growing environment (Wood 1995; Landis *et al.* 1992). This produces seedlings that have adapted, both morphologically and physiologically, to high humidity conditions. However, these conditions are rarely found on open canopy forest sites. Incidence of increased wind gusts, incident light and daytime temperature, as well as a decrease in early morning and night temperatures, and humidity levels have been reported (Balisky and Burton 1995; Jordan and Smith 1995; Marsden *et al.* 1996; Kimmons 1997; Man and Lieffers 1997). To reduce the high transpirational demand on newly planted seedlings caused by low humidity levels, seedlings must either be planted where humidity levels are higher, such as under closed or partially-closed canopies (Marsden 1996), or morphologically or physiologically adapted to tolerate low humidity conditions. It is possible that such adaptations could be developed by growing seedlings under low humidity levels during their early and rapid growth phases in the nursery.

Humidity plays a crucial role in the nursery production of conifer seedlings (Landis *et al.* 1992; Wood 1995). Exceedingly high humidity levels can cause excess water vapor to condense on seedlings or container walls, providing an ideal environment for the growth of opportunistic or pathogenic organisms such as mosses, algae, liverworts, fungi, molds or insects (Landis *et al.* 1989a, 1989b, 1992; Elad *et al.* 1996). Excessively low humidity produces a biophysical stress, modifying the water relations of plants by creating a high vapor pressure deficit (VPD) around the leaves (Gaffney 1978; Nonami *et al.* 1990; Assmann and Gershenson 1991). The high water vapor concentration difference between the atmosphere and mesophyll tissues causes the plant to lose water to the atmosphere at high rates, placing a potential water stress on the plant (Nonami *et al.* 1990; Assmann *et al.* 1991; Larcher 1995). Excessive transpiration can lead to stomatal closure, which in turn can limit the transport of water and mineral nutrients through a plant (Collatz *et al.* 1991; Weiser and Havranek 1996).

Although humidity is acknowledged as an important factor regulating the growth and development of plants (Krizek *et al.* 1971; Morrison Baird and Webster 1978; Gaffney 1978; van de Sanden 1985) limited research exists on how it influences the morphology

or physiology of conifer seedlings. It is also poorly understood what adaptations, if any, seedlings can develop when growing under a continually high transpirational demand. Investigating alternative growing methods that can be used to adapt seedlings for future growth on low humidity sites is the basis for this study.

The objective of this study was to determine if rearing white spruce seedlings under various humidity levels would alter the initial growth, morphology, quantitative cuticular wax production, frost tolerance and drought resistance of the seedlings. As well, to determine if treatment effects were preserved after flushing and growing the seedlings under subsequent conditions of low and high humidity.

Materials And Methods

Germination and early growth

White spruce seeds (seedlot # DS 70-9-5-91) from Slave Lake, Alberta, at an elevation of 670 m were used in this study. A peat:vermiculite (3:1) soil mixture was adjusted to a pH of 5.5 with dolomite lime and autoclaved at 160°C for 60 min. Nine Superblock 160's (60 ml cavities) were acquired from Beaver Plastics (Edmonton, AB), sterilized, and filled with the soil mixture to the recommended density of 0.09 g/ml (Wood 1995). Three white spruce seeds per cavity were sown, and thinned to one seedling per cavity after germination. For germination, the Styroblocks were randomly placed in three growth chambers (three Styroblocks per chamber), and maintained under the same conditions (Table 2.1). Three weeks after seeding, the growth chambers were set to the recommended "early growth phase" environmental conditions (Table 1.1), and maintained for approximately two weeks. At five weeks the Styroblocks were rearranged between the chambers and the chambers set to the experimental treatments as outlined below. Block dry-down weights (weighed to 80% dry-down weight to determine the frequency of watering), soil pH, and soil salinity measurements were made every three to four weeks to remain within recommended guidelines (Wood 1995). Seedlings were fertilized according to growth stage following the recommendations of Wood (1995).

Experimental treatments

The three growth chambers used in this experiment were maintained at the same environmental conditions, with the exception of the relative humidity (RH). At the beginning of week five, each of the three chambers was set to either high (80%), medium (50%), or low (30%) RH. Seedlings were grown under these RH conditions until approximately week 21, for a total of 16.5 weeks. Because vapor pressure deficit (VPD) is believed to be the humidity parameter detected by plant guard cells (Assmann *et al.* 1991), and some tree nurseries measure VPD to determine evapotranspirational demand on the seedlings, the experimental humidity treatments were converted to VPD values (Table 2.2). Container tree nurseries recommend maintaining the VPD below 1.0 kPa, as above this point the evapotranspirational demand on the tree is high, likely resulting in closure of the stomata and a decrease in the rate of photosynthesis (Landis *et al.* 1989).

Seedling hardening

Seedlings were removed from the experimental treatments during week 21 of growth. At that time, atmospheric conditions were changed (Table 1.1) to inhibit shoot elongation and induce bud set. For both the 50% and 80% RH treatments, the RH was reduced to approximately 45-50%. However, the 30% RH seedlings were maintained at 30% RH. After the seedlings were hardened off, they were removed from the Styroblocks, bundled in groups of 20, and the soil plugs wrapped in plastic. Any seedlings showing signs of

mold or disease were discarded. Seedlings were placed in wax-lined boxes and stored at 3±1 °C (Macey and Arnott 1986; van den Driessche 1991a) for eight weeks before further testing.

Table 2.1. Air temperature, photosynthetically active radiation (PAR), photoperiod and humidity values during seedling growth from germination to the hardening-off stage. The three values in the humidity column during the early and rapid growth represent the three experimental treatment chambers. All values are means ± SD.

Seedling Growth Phase	Duration weeks	Air Temperature °C Day	Night	PAR $\mu mol \cdot m^{-2} \cdot s^{-1}$	Photoperiod $h \cdot day^{-1}$	RH %
Germination	0-3	20.0±2.2	18	380±20	18	78±5.3
Early Growth	3-10	19.9±1.2	18	375±35	18	31.0±2.8 48.7±3.3 80.4±0.9
Rapid Growth	10-19	20.0±.98	18	375±27	18	31.0±2.8 48.7±3.3 80.4±0.9
Bud Initiation	19-21	12.0±1.1	10	380±18	14	33.1±4.0 47±8.6
Stem Finishing	21-24	12.0±1.1	10	370±31	14	33.1±4.0 47±8.6

Table 2.2. Comparison of relative humidity (RH) to vapor pressure deficit (VPD) for the three experimental treatments.

Treatment	Temperature °C *Day*	*Night*	RH (%) *Day*	*Night*	VPD (kPa) *Day*	*Night*
30% RH	20	17	30	30	1.63	1.40
50% RH	20	17	50	50	1.17	0.95
80% RH	20	17	80	80	0.47	0.45

Seedling Measurements

Growth

Seedling height (n=45) was non-repetitively measured approximately every two weeks, from six to 26 weeks after sowing. Measurements were made from the top of the soil plug to the tips of the terminal needles (Thompson 1985). Root collar diameter (RCD) measurements commenced after week 10, and were made approximately 0.5 cm above the soil plug (Thompson 1985).

Frost tolerance

Frost tolerance measurements were conducted between weeks 20 and 21 on seedlings that were still unhardened. Seedlings were tested at temperatures of -6, -8, -10, -12 and -14°C with n=6 seedlings per temperature per treatment. Frost tolerance was measured using a Fisher model C33 conductivity meter (Fisher Scientific, Ontario, Canada), and the electrolyte leakage method (Glerum 1985). For this experiment, the distal 4-5 cm of the uppermost shoots were used, and total electrolytes were expressed by placing the shoots in test tubes filled with de-ionized water, and immersing the tubes into a boiling water bath for 60 min.

Epicuticular wax

Once seedlings reached 26 weeks, approximately 10 seedlings from each treatment were randomly selected, the shoots rinsed with de-ionized water and immersed in liquid nitrogen to facilitate removal of needles. The needles were then freeze-dried and stored at -20°C until the time of analysis. To quantify the epicuticular wax content, approximately 20 mg (13-18 needles) were weighed out then placed in a glass test tube. Chloroform (3.0 ml) was added to the tube (Walton 1990; Gulz 1994), then it was vortexed for 30 s to chemically remove the epicuticular wax. The chloroform was decanted through two layers of cheesecloth to prevent needle loss, then the needles were dried for four days and re-weighed.

Bud flush

Sixty seedlings from each treatment were removed from cold storage and divided into two groups, with 30 seedlings per treatment per group. One seedling from each treatment was planted into a 4 L pot containing the peat:vermiculite soil mixture, for a total of three seedlings per pot, and 30 pots per group. Seedlings were placed under similar conditions (Table 1.4) with the exception of the RH. The first group was subjected to 42% RH, and the second 73% RH. All seedlings were monitored every three days for signs of terminal and lateral bud flush. Bud flush was defined as having at least 1.0 mm of new needle tissue exposed, and was recorded for 30 seedlings per treatment with no sub-samples. Results were recorded as the cumulative percentage of seedlings within a treatment that had flushed their terminal, or at least one lateral bud from the time of potting.

Needle and shoot morphology

Needle density and needle length was measured in three groups of seedlings (n=15 seedlings per treatment). The first group consisted of seedlings that were directly removed from cold storage, and represented treatment effects on first year seedlings. The second group contained seedlings that had flushed and grown for eight weeks under 42% RH representing, and represented treatment effects on seedlings subsequently grown under lower RH conditions. The third group were seedlings were flushed and grown under 73% RH for eight weeks, and represented treatment effects on seedlings that were subsequently grown under higher RH conditions. Measurements for density and length were made on needles that grew at approximately half way up the shoot, and did not include needles that were either still expanding or that might have been affected by hardening off. Needle density was determined as the number of needles within a 1.0 cm section of stem.

Drought resistance

Drought resistance was determined in seedlings that were flushed and grown under 73% RH. There was not enough new shoot tissue to allow comparable measurements on seedlings flushed and grown under 42% RH. Two methods were used to determine water stress level, soil water availability and seedling water potential. Soil water availability was calculated as the percent dry-down weight of the pots the seedlings were grown in. The pots (n=10) were watered to saturation, left for 30 min. to stop dripping, then weighed. This weight was taken as 100% pot saturation, and used as a baseline to determine percentage dry-down weights. As pots dried down during the experiment, they were re-weighed and the weight stated as a percentage of pot saturation. The second method involved measuring the shoot xylem water potential, using a Scholander pressure chamber (PMS Instrument Co., Corvallis, OR). Six seedlings per treatment were measured (non-repetitively) throughout the drought period, until the seedlings reached approximately -2.0 MPa, when this suggested that seedlings were experiencing a severe water stress. Drought effects on seedling photosynthesis, transpiration, and stomatal conductivity were measured using an LCA-4 infra-red gas analyzer (IRGA, Analytical Development Company Ltd., Hoddesdon, U.K.). The measurements (n=6 seedlings per treatment) were made throughout the drought experiment, until water potentials of the seedlings reached below -2 MPa, or approximately 30% pot saturation weight. At that time, the seedlings were re-watered, and their recovery from water stress (measured as the return to pre-drought water potential levels) was measured three and five days after watering.

Gas exchange

Net assimilation (NA, or photosynthesis) , transpiration (E) and stomatal conductance (g_s)were measured in seedlings flushed under 42% and 73% RH conditions. Sample size

was 6 seedlings per treatment, with four measurements per seedling being averaged. Measurements per individual seedling were conducted on the same shoot, and recordings were made at 30 s intervals after photosynthesis levels had stabilized. Gas exchange was measured using an IRGA fitted with a conifer cuvette. Leaf area was determined from scanned images of the needles, and calculated using Sigma Scan Pro 3.0 software from Jandel Scientific.

Results

Growth

By week 8, low and moderate humidity levels reduced seedling height growth significantly (P=0.04), compared to seedlings from the 80% RH treatment (Figure 2.1a). At week 14 (eight weeks of RH treatment), significant height differences were found between the 30% and 50% RH treatments. Treatment effects on RCD followed the same general trend as the height data (Figure 2.1b). Significant (p=0.020) differences in root collar diameter between the 80% RH and the 30% and 50% RH seedlings were not apparent until week 12, when the 80% treatment seedlings showed a larger diameter. This trend continued until week 26. No significant differences in RCD were found between the 30% and 50% RH seedlings throughout the experiment.

Frost tolerance

In general, unhardened seedlings from all treatments were found to lose hardiness between -6 and -8 °C (data not shown). However, ANOVA results indicated no significant treatment (humidity), or treatment and temperature interaction effects on the frost tolerance of the seedlings.

Epicuticular wax

Seedlings reared under 50% RH produced needles that were significantly lighter than those reared under 80% RH (Table 2.3). The 30% RH needles produced marginally greater (p=0.056) quantities of wax compared to those reared under the 80% RH conditions, when expressed on a per gram of dried tissue basis. There was a clear trend in the quantity of epicuticular wax produced when expressed as a percent of needle dry weight (Table 2.3), with decreasing quantities associated with increasing humidity. However, these differences were not statistically different.

Table 2.3. Needle epicuticular wax content from the humidity treated seedlings. Means (± SE n=10 seedlings) with the same letter within a column are not statistically different based on a Tukey's test.

	Mean Dry Weight (mg)	Mean Wax Produced (mg/g dried needles)	Wax Quantity (% needle dry weight)
30% RH	1.28 (.31) **ab**	16.2 (2.95) **a**	0.77 **a**
50% RH	1.00 (.22) **a**	14.3 (3.30) **ab**	0.71 **a**
80% RH	1.54 (.39) **b**	12.8 (1.83) **b**	0.62 **a**

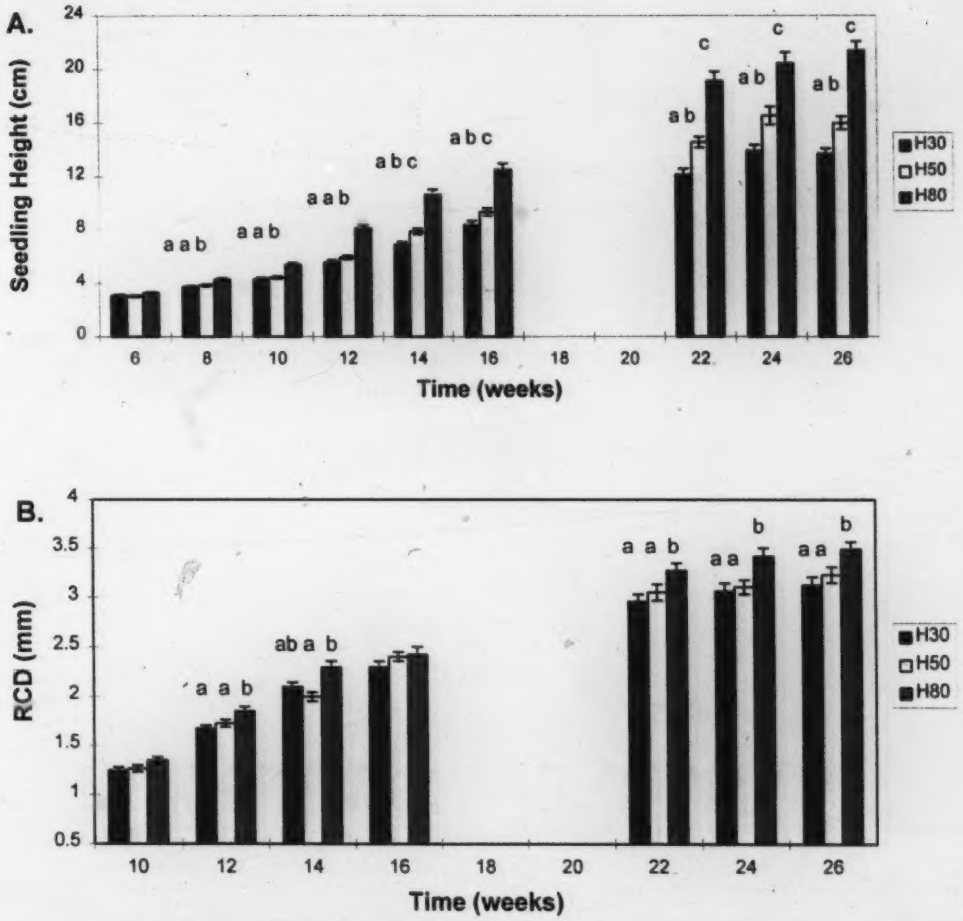

Figure 2.1. Mean shoot height (**A**) and RCD (**B**) growth of seedlings reared under 30% (H30), 50% (H50) or 80% (H80) RH. Means (± SE) with the same letter are not significantly different at the 0.05 level, based on a Tukey's test; n=45 per treatment.

Bud flush

Table 2.4 lists the two experimental conditions under which seedlings were flushed and grown following cold storage. Figure 2.2a shows that under conditions of 74% RH, seedlings from all three treatments flushed their terminal buds nine days after potting, but the 30% RH seedlings flushed a greater proportion. By day 18 all seedlings from the 30% and 50% treatment had flushed, while those from the 80% treatment required an additional six days. Under 42% RH conditions, seedlings from all treatments took longer to flush compared to the same seedlings flushed under 74% RH, with the 30% RH seedlings first flushed on day 12, and the 50% and 80% RH seedlings required an additional three days. By day 18 most of the 30% RH seedlings had flushed, compared to day 24 for the 50% and 80% seedlings.

Lateral bud flush (Figure 2.2b) under 74% RH followed the same general trend as that of the terminal buds. Seedlings from all treatments showed signs of lateral bud flush by day nine, but again, a greater proportion of flushing seedlings were from the 30% RH treatment, followed by the 50% and finally the 80% RH treatment. By day 15, all seedlings from the 30% and 50% treatments completed their lateral bud flushing, but seedlings from the 80% RH treatment required an additional three days. Under the 42% RH conditions, lateral bud flush occurred for all three treatments by day 9, three days later compared to seedlings flushed under 74% RH.

Table 2.4. Experimental RH conditions for the bud flush experiment.

Environment	RH (%)	Air Temperature (°C day/night)	PAR ($\mu mol/m^2/s$)	Photoperiod (h)
Low Humidity	42.2±2.2	21/18	385±25	18
High Humidity	73.7±3.4	21/18	390±10	18

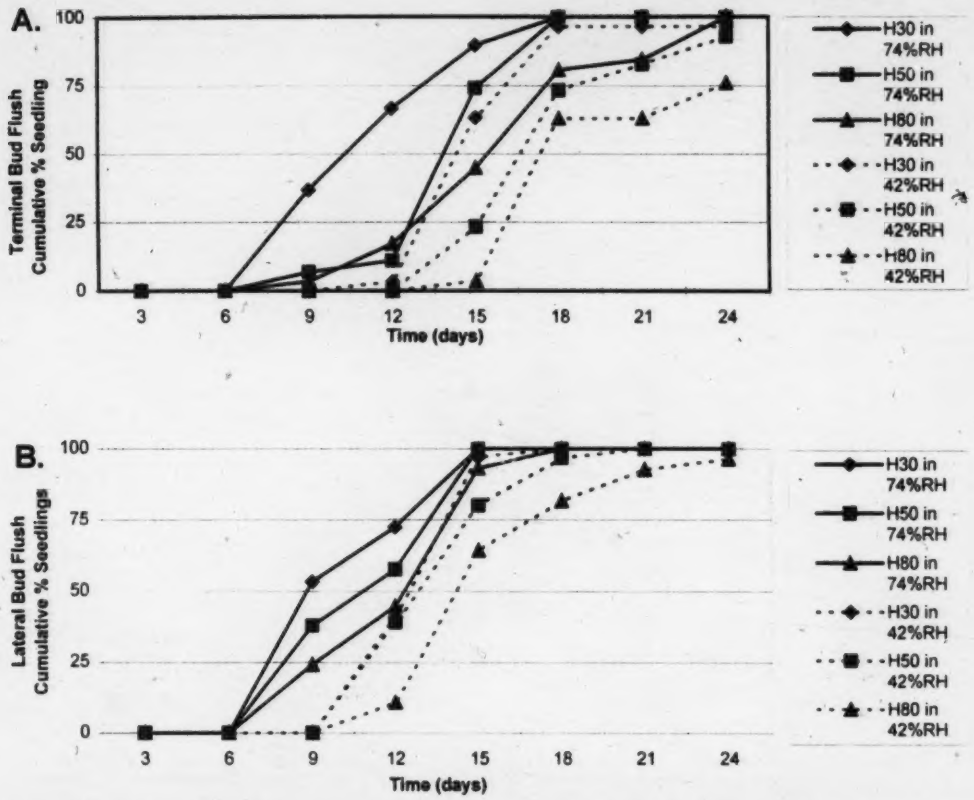

Figure 2.2. Effects of humidity on terminal (**A**) and lateral (**B**) bud flush of white spruce seedlings. The seedlings were originally grown under 30% (H30), 50% (H50) or 80% (H80) RH conditions, set dormant, then subsequently flushed under 42% RH and 74% RH. N=30 seedlings per treatment.

Needle and Shoot morphology

In the sample of seedlings removed from cold storage, the 30% and 50% RH seedlings were similar in height, but the 80% RH seedlings were significantly taller (Table 2.5). After flushing under 74% RH, there were no significant differences in final seedling height between all three treatments. However, there were significant ($p<0.001$) treatment effects on both the length of new shoots, and the new to old shoot ratio. The 80% RH seedlings had greater RCD after hardening compared to the 30% and 50% seedlings (Table 2.5). When grown under 74% RH, the 30% RH seedlings had a smaller RCD ($p=.040$) compared to seedlings from the 50% and 80% RH treatments (Table 2.5).

Table 2.5. Shoot height and RCD of seedlings from the three humidity treatments. Hardened seedlings refers to those removed from cold storage, while flushed seedlings refers to those grown under 74% RH for eight weeks. Means (± SE) with the same letter within a column are not statistically different at the 0.05 level based on a Tukey's test; n=30 seedlings per treatment.

	Hardened Seedlings	Seedlings Flushed under 74% RH	Absolute New Growth	% New Growth Increase
Height (cm)				
30% RH	16.7 (1.08) **a**	29.5 (1.41) **a**	15.7 (1.14) **a**	52.9 (1.70) **a**
50% RH	18.7 (1.70) **a**	28.6 (1.13) **a**	11.8 (0.81) **b**	40.9 (2.06) **b**
80% RH	25.3 (2.26) **b**	30.5 (1.45) **a**	8.06 (1.11) **c**	26.0 (2.30) **c**
RCD (mm)				
30% RH	2.96 (0.074) **a**	5.17 (0.158) **a**	2.21 **a**	40.9 **a**
50% RH	3.05 (0.082) **a**	5.71 (0.168) **b**	2.66 **a**	45.1 **a**
80% RH	3.37 (0.068) **b**	5.91 (0.237) **b**	2.54 **a**	41.9 **a**

First year seedlings showed a significant ($p<0.001$) treatment effect on needle length, with the 30% RH seedlings having the shortest needles and the 80% RH seedlings the longest (Table 2.6). After flushing under 42% RH, the reverse trend was observed, with the 80% RH seedlings producing significantly shorter needles compared to the 30% and 50% RH seedlings. However, when the seedlings were flushed under 74% RH, no significant differences in needle length were found. For needle density, lower humidity produced significantly ($p=.0002$) denser needles in the 30% and 50% RH seedlings, when measured in seedlings removed from cold storage (Table 1.6). After flushing under 42% RH, the reverse trend was seen, with the 30% and 50% RH seedlings producing significantly ($p<0.001$) fewer needles than the 80% RH treatment seedlings. When

seedlings were flushed under the 74% RH, the 30% and 50% RH seedlings also had significantly lower needle density than the 80% RH seedlings.

Table 2.6 Needle length and density for seedlings removed from cold storage (hardened), and for seedlings flushed under either 42% or 74% RH. Means (± SE) with the same letter within a column are not statistically different at the 0.05 level based on a Tukey's test; n=30 seedlings per treatment.

	Hardened	Flushed under 42% RH	Flushed under 74% RH
Needle Length (cm)			
30% RH	1.65 (0.074) **a**	2.12 (0.059) **a**	2.18 (0.10) **a**
50% RH	1.96 (0.065) **b**	1.93 (0.080) **a**	2.13 (0.050) **a**
80% RH	2.21 (0.070) **c**	1.60 (0.073) **b**	1.98 (0.051) **a**
Needle Density (needles cm^{-1} stem)			
30% RH	33.0 (1.78) **a**	24.7 (1.53) **a**	16.7 (1.08) **a**
50% RH	29.5 (1.12) **a**	27.9 (2.13) **a**	18.7 (1.40) **a**
80% RH	23.5 (1.24) **b**	34.3 (2.04) **b**	25.3 (2.20) **b**

Drought resistance

There were no significant humidity effects on net assimilation or transpiration immediately prior to, during, or in recovery from drought (data not shown). Any apparent differences that were found in seedling water potentials were due to a blocking effect, not a treatment effect. There were significant humidity effects on seedling water potential levels when seedlings experienced a severe drought (30% pot saturation, Figure 2.4). The 30% RH seedlings maintained significantly ($p=.026$) higher water potentials compared to the 50% and 80% RH seedlings. Seedlings from all treatments recovered to similar water potential levels three and five days after being re-watered.

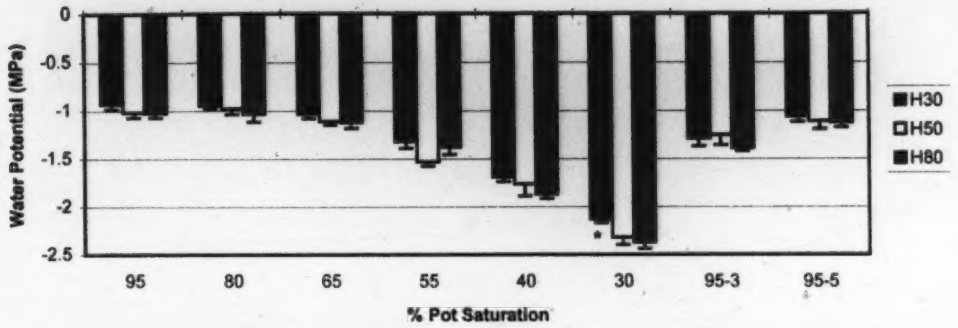

Figure 2.3 Shoot xylem water potentials in 21-week-old seedlings during a water stress experiment. Recovery from drought is indicated three days after re-watering (95-3) and five days after watering (95-5). Values are mean (± SE); n=6 seedlings per treatment. * indicates significance at the 0.05 level based on a Tukey's test.

Gas exchange

There were no significant differences in NA between seedlings from the three humidity treatments that were flushed and grown under 74% RH (Figure 2.3a). Small but significant ($p=0.046$) treatments differences in NA occurred between seedlings measured under the 43% RH conditions, with the 30% RH seedlings maintaining the highest NA values, followed by the 50% and 80% RH seedlings. All NA levels were significantly higher when measured under the 74% RH conditions compared to the 42% RH.

For seedlings flushed under 74% RH, the 80% RH seedlings maintained significantly lower E levels compared to the 30% and 50% RH seedlings (Figure 2.3b). Under the 42% RH conditions, the 30% RH seedlings had significantly higher transpiration values compared to the 50% RH seedlings, which in turn were significantly higher than the 80% RH seedlings. When measured under 42% RH, the 30% and 50% RH seedlings had significantly higher g_s values compared to the 80% RH seedlings (Figure 2.3c). However, when measured on day 17, the 80% RH seedlings had the highest stomatal conductance values.

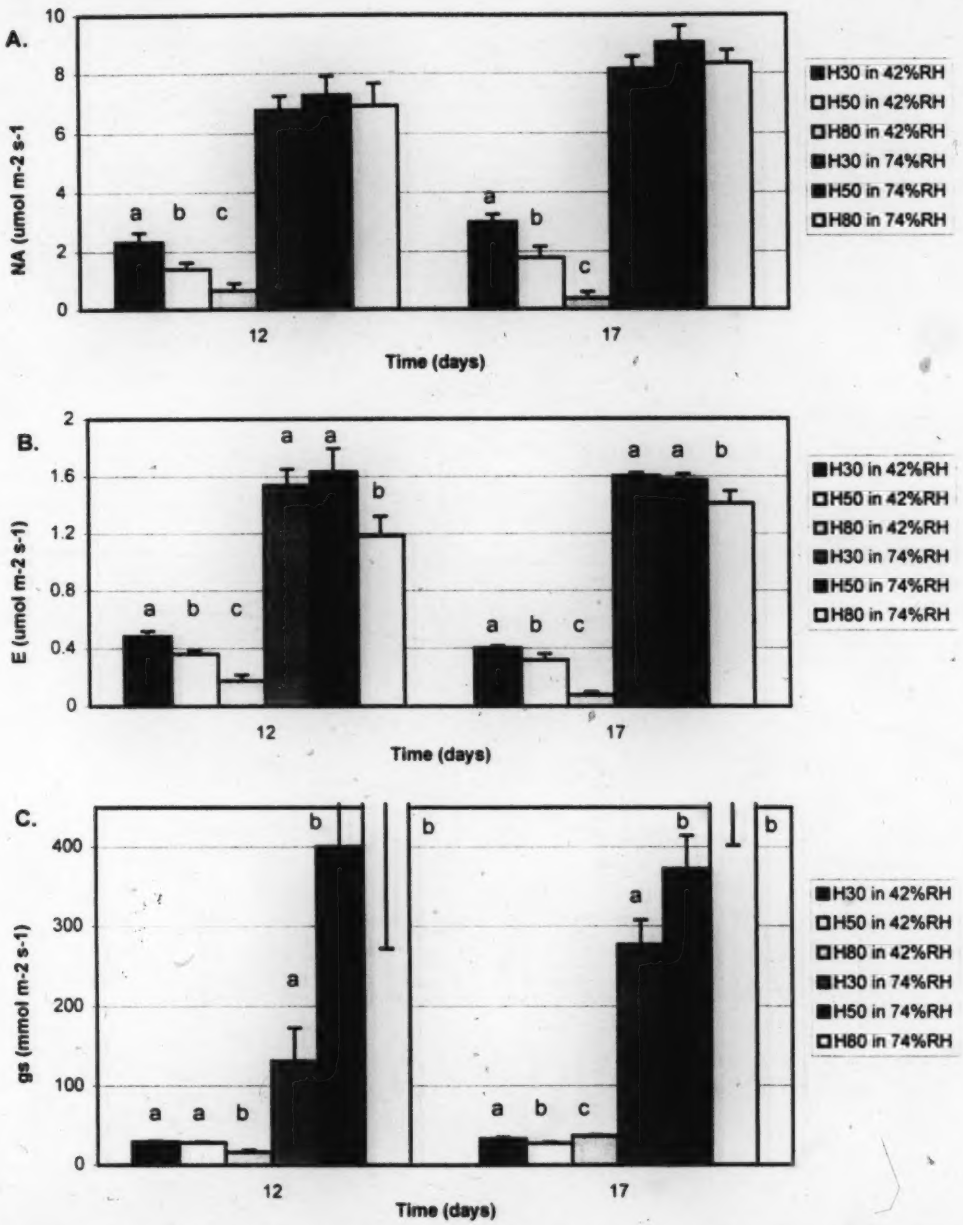

Figure 2.4. Net assimilation (**A**), transpiration (**B**) and stomatal conductance (**C**) of white spruce seedlings initially grown under either 30% (H30), 50% (H50) or 80% (H80) RH, then subsequently flushed and grown under 42% or 74% RH. Measurements were made 12 and 17 days after potting seedlings. Values are means (± SE), and n=6 seedlings per treatment.

Discussion

In this experiment, humidity had a significant influence on the growth white spruce seedlings. These results agree with the observations of Krizek *et al.* (1971) and Morrison Baird and Webster (1978). Plants respond to high transpirational loss by closing their stomata, even if there is an adequate water supply to the roots (Grantz and Meinzer 1990; Larcher 1995; Percival *et al.* 1996). Stomatal closure in turn can lead to decrease rates of photosynthesis and transpiration (Nonami *et al.* 1990; Assmann *et al.* 1991; Wieser and Havranek, 1996). Together these factors could account for the lower growth found in the low and moderate humidity grown seedlings compared to the high humidity seedlings. However, reducing RH from 50% to 30% did not result in a further decrease in RCD. Therefore, maintaining greenhouse VPD below 1.0 kPa, as is currently recommended (Landis *et al.* 1989), may produce seedlings with larger stem diameters.

Waxes which are soluble in chloroform represent the greatest barrier to transcuticular water vapor diffusion (Hadley and Smith 1990). The amount of chloroform-removed wax, as a percent of seedling weight, was slightly higher but still in agreement with previously published values for white spruce (Tulloch 1987). Wax quantity per gram of dried tissue was higher in the 30% compared to the 80% RH needles, but within similar ranges reported by Guenthardt (1984), and Cape and Percy (1993). Since large differences in wax quantity were not found between the three treatments, it is unlikely that low humidity levels per se induced a greater quantitative production of epicuticular wax for white spruce seedlings. However, further studies on how low humidity might influence the qualitative production of these waxes is recommended. The 50% RH seedlings had the lowest weight per needle, but significantly longer needles compared to the 30% RH seedlings. This suggests that lower humidity levels will modify needle anatomy.

In the current study lower humidity levels (42% RH) after flushing delayed the onset of seedling lateral or terminal bud flush by approximately 3-9 days. These results have two implications. First, that humidity affects the initial timing of bud flush. Second, the extent to which humidity modifies the timing of bud flush depends on the humidity levels present before the seedlings set bud. In a literature review of the effects of environmental conditions on bud dormancy, Lavender (1985) acknowledges soil moisture, photoperiod, temperature and nursery cultural regime as major factors that can contribute to delayed seedling bud flush, but did not discuss the role of humidity. It is possible that atmospheric water stress may be a factor which influences the timing of bud flush, by placing a high transpirational demand on the seedlings.

Morphological characteristics of plants vary in response to the environmental conditions under which they are grown (Kimmons 1987; Leverenz and Hinckley, 1990; Reich *et al.* 1996; Sprugel *et al.* 1996; Man and Lieffers 1997). This study found that humidity is a factor in determining seedling shoot and needle morphology, as 30% RH produced

needles that were both shorter and more densely spaced than the higher RH-grown seedlings. However, when these seedlings were subsequently flushed under 42% RH, the 30% RH seedlings produced longer needles compared to those from the higher humidity treatments. This suggest that the humidity conditions before bud set will modify the morphology of the subsequent year's needles. A possible reasons for this is that the 30% RH seedlings became physiologically adapted to lower humidity levels, allowing them to have relatively greater growth compared to seedlings reared under higher humidity conditions, under similar subsequent conditions. Physiological mechanisms of adaptations could include modified stomatal sensitivity, increased seedling water use efficiency, or possibly osmotic adjustment.

Although the 30% and 50% seedlings were initially shorter than the 80% RH seedlings, subsequently growing them under 74% RH resulted in overall similar seedling heights, but smaller RCD in the 30% RH seedlings. This suggests that initial humidity effects on shoot length are not persistent under higher humidity levels, and that the focus of the 30% RH seedling growth was on shoot elongation rather than radial growth. It is unknown if this trend would continue for a third growing season, or that any physiological benefits incurred through the initial adaptation of seedlings to low humidity, would remain after the second growing season.

When water is withheld from a plant, drought stress occurs slowly, allowing plants time to make water-conserving physiological adjustments such as altered stomatal sensitivity, increased osmotic potential, or increased water use efficiency (Zwiazek and Blake 1989; Koppenaal et al 1991; Zwiazek 1991; Edwards and Dixon 1991). Since these parameters were not measured throughout this experiment, it is unclear how the low humidity seedlings were able to maintain higher water potentials during the severe drought. Seedling recovery from drought was measured three days after re-watering, and no treatment differences were found. However, it is possible that treatment effects on seedling recovery might have occurred within the first 48 hours after re-watering. Increasing the frequency of measurements after re-watering would have addressed this issue.

Gas exchange parameters were reduced when measured for the lower, compared to the higher, humidity grown seedlings. Under 74% RH conditions, stomatal conductance values for the 30% RH seedlings were significantly lower, while photosynthesis and transpiration values were similar or higher to the other two treatments. Under the 42% RH conditions, higher photosynthesis, transpiration and stomatal conductance values were found for the 30% compared to the 50% and 80% RH seedlings. These results suggest that, 1) lower RH conditions reduces seedling photosynthesis, transpiration and stomatal conductance, 2) low RH conditions during initial seedling production may physiologically adapt seedlings to maintain higher gas exchange rates under subsequent conditions of low humidity. The interactive effects of light, humidity and temperature may induce different stomatal responses in certain species (Nonami *et al.* 1990), but changes in stomatal sensitivity should also be considered as possible adaptation

mechanisms. Further studies to identify the specific physiological adaptation mechanisms displayed in the current study are required.

Conclusions

Based on the following observations it is concluded that rearing white spruce seedlings under lower than the currently recommended RH conditions will improve planting stock quality, provided that the outplanting site is characterized by low RH conditions. These observations are: 1) that white spruce seedlings reared under low relative humidity conditions did not show significant height differences, compared to seedlings reared under higher RH conditions, after one season of field growth; 2) needle density was reduced while needle length was increased after growing the low RH seedlings under subsequently low RH conditions; 3) during a sever water deficit, low RH seedlings showed less water stress, and recovered faster to baseline water potential levels, than high RH reared seedlings; 4) under subsequently low RH conditions, low RH-reared seedlings flushed their terminal buds faster that high RH reared seedlings.

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Chapter III: Periodic Low Temperature Effects on White Spruce (*Picea glauca*) Physiology

Introduction

During container seedling production, greenhouse air temperature is monitored closely, as it directly influences the growth and development of plants (Hellmers *et al.* 1970; Brix 1971; Landis *et al.* 1992; Wood 1995). In general, for every increase in 10°C there is a 2-3 fold increase in the rate of plant biochemical reactions (Raven *et al.* 1992). However, maintaining warm temperatures to favor high growth rates may not be practical during winter months as the heating costs to the nurseries become too high (Draper and Hawkins 1989). Exposing plants to chilling affects their growth and development, the extent of which depends on the temperature, the duration of the exposure, and the sensitivity of the species (Graham and Patterson 1982; Wang 1982; Markhart 1984; Alberdi and Corcuera 1991; Anderson *et al.* 1994). In general, northern conifer species, including white spruce, are relatively tolerant to chilling temperatures and are able to grow at temperatures below 10°C, although this growth is very slow (Landis *et al.* 1992).

Most North American tree nurseries maintain greenhouse temperatures between 17° to 25°C during the initial 18-20 weeks of seedling growth (Landis *et al.* 1992). This is to promote rapid shoot elongation, needle production and root development during their early and exponential growth phases (Landis *et al.* 1992; Wood 1995). Many northern latitude tree nurseries seed their containers during the winter months (Draper and Hawkins 1989), and therefore heating costs to the nurseries are high due to low outside temperatures. Reducing these costs by decreasing the greenhouse temperature from 16° to 10°C over an extended time period can save approximately 24% of the heating costs (Landis *et al.* 1992). Draper and Hawkins (1989) determined that germinating seedlings under a 20°/11°C (12h:12h) temperature regime saved up to 50% of heating costs when compared to a 20°/20°C (12h:12h) regime. They found no significant treatment effects on seedling morphology. Periodically reducing the greenhouse temperature for a short duration may, therefore, be an important cost saving strategy.

Periodic temperature fluctuations reflect what some early spring-planted seedlings would experience in boreal forest cut-blocks, where low evening and morning temperatures are followed by high afternoon temperatures (Balisky and Burton 1995; Jordan and Smith 1995). This temperature variation is due to the loss of protective tree canopy that can no longer trap long wavelength energy released and reflected from the ground and understory plants (Balisky and Burton 1995; Jordan and Smith 1995). Previous reports reviewed specific changes to plants as a result of acclimation to low temperatures (Levitt 1980; Alberdi and Corcuera 1991; Anderson *et al.* 1994, 1995). However, most reports focused on how plants acquire an increased level of frost or chilling resistance as a result

of preconditioning to low temperatures (De Yoe *et al.* 1988; Anderson *et al.* 1994, 1995; Zhao *et al.* 1995). Few studies examined the effects of low temperature exposure on other forms of stress resistance, such as drought resistance. Van den Driessche (1991) found that reducing nursery greenhouse temperatures increased survival for lodgepole pine and white spruce seedlings after outplanting under xeric conditions, and increased the relative growth rate of Douglas-fir seedlings after outplanting. Therefore, incorporating low temperatures into nursery cultural practices could increase the overall stock quality of conifer seedlings.

The main objectives of this study were 1) to examine the effects of periodic chilling on the growth, morphological characteristics and stress resistance of white spruce [*Picea glauca* (Moench) Voss] seedlings, and 2) to determine if the initial treatment effects on growth or morphology were persistent in field planted seedlings after the second growing season.

Materials And Methods

Germination and early growth

White spruce seeds were obtained from the Pine Ridge Forest Nursery (Smoky Lake, Alberta, seedlot # DS 70-9-5-91) were from Slave Lake, Alberta, at an elevation of 670 m. A peat:vermiculite (3:1) soil mixture was adjusted to a pH of 5.5 with dolomite lime and autoclaved at 160°C for 60 min. Superblock 160 containers (60 ml cavities, Beaver Plastics, Edmonton, Alberta) were sterilized, filled with the soil mixture to a density of 0.09 g/ml (Wood 1995) and seeded. All Styroblock containers were placed in one growth chamber for germination and early growth (see Table 3.1 for environmental conditions). Soil conductivity, pH and block dry-down weights were monitored approximately every two to three weeks and adjusted as needed. Seedlings were fertilized according to Wood (1995).

Table 3.1. Environmental conditions for the five stages of seedling growth during the temperature experiment.

Growth Phase	Air Temp. (°C)	RH (%)	PAR ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Photoperiod (h)
1. Germination				
warm growth room:	20.0 ± 2.2	78 ± 5.3	380 ±20	18
2. Early Growth				
5°C chamber:	5.0 ± 0.52	75 ± 4	390 ± 16	18
warm growth room:	20.6 ± 1.5	64 ± 9.1	375 ± 35	18
3. Rapid Growth				
5°C chamber:	5.1 ± 0.37	73 ± 8	395 ± 10	18
warm growth room:	20.9 ± 1.2	67.5 ± 11	375 ± 33	18
4. Bud Initiation				
5°C chamber:	5.1 ± 0.4	74 ± 5	395 ± 10	14
warm growth room:	17.2 ± 3.6	61 ± 8.6	380 ± 20	14
5. Stem Finishing				
warm growth room:	12.0 ± 11	47 ± 8.6	370 ± 30	14

Temperature treatments

Temperature treatments were initiated five weeks after seeding (during the early growth phase) and ended during week 21 (stem finishing), for a total of 16.5 weeks. The experiment was a randomized block design with four treatments (three blocks per treatment) that varied their frequency of seedling exposure to 5°C. The four treatments were: (1) T1/2: one day at 5°C, followed by two days under warm conditions; (2) T1/3: one day at 5°C, followed by three days at warm conditions; (3) T1/4 one day at 5°C, followed by four days at warm conditions; and (4) TC: the control seedlings, continuously grown under warm conditions. The exposure of seedlings to warm and cold cycles was continued for 16.5 weeks. Environmental conditions for the warm and cold growth rooms during seedling development are shown in Table 3.1.

Measurements

Growth

Seedling height (n=45, 15 seedlings per block per treatment) was non-repetitively measured every two weeks between week five and 25, following germination. Measurements were made between the top of the soil plug and the tips of the terminal needles (Thompson 1985). Starting at week 10, root collar diameter (RCD) was measured in 45 seedlings for each temperature treatment, approximately 0.5 cm above the soil plug (Thompson 1985).

Drought resistance

Response to drought was measured on 21-week-old seedlings. Seedlings from each of the four treatments were kept in their original Styroblock cavities, and maintained under warm growing conditions (Table 3.1) for the duration of the experiment. All seedlings were well-watered the week prior to the drought experiment. On day one, mid-day shoot water potential measurements were made using a Scholander pressure chamber (PMS Instrument Co., Corvallis, Oregon), then water was withheld from the seedlings for nine days to induce a water-deficit stress. On days seven and nine, mid-day shoot water potentials were measured, and on day ten the seedlings were re-watered to soil saturation. Water potentials were once again measured on day 11 to determine seedling recovery from drought. Sample size was nine seedlings (three seedlings per block per treatment).

Frost Tolerance

Frost resistance was measured on 18-week-old, unhardened seedlings (n=6 seedlings, 2 shoots per block per treatment), using the frost induced electrolyte leakage methods of Glerum (1985). Changes to these methods were as follows: lateral shoots located closest to the terminal leader were used instead of the terminal shoot; and seedling shoots were immersed in a boiling water bath for one hour to kill the shoots. Testing temperatures of -3, -5, -7, -9, -11, -13 and -15°C were reached using a programmable freezer. The freezer

temperature was reduced by 2°C per hour, remaining at the testing temperature for one hour after reaching the desired temperature. Electrical conductivity of the frozen and killed shoot tissues was measured using a conductivity meter (model #32, Fisher Scientific, Edmonton, Canada). Final electrolyte leakage measurements were expressed on a relative scale (index of tolerance), with low values reflecting high tolerance to that temperature, and high values reflecting loss of frost tolerance at that temperature.

Root growth capacity

Thirty seedlings from each treatment were removed after cold storage at 2-4°C for four weeks (Van den Driessche 1991). Four seedlings, one from each treatment, were potted into a 14-inch square pot. All pots (n=30) were placed in a growth chamber at a 10°C with an 18-h photoperiod and 50% humidity. After 21 days, the seedlings were removed from the pot, and the number of new white roots was recorded according to two size classes: 0-0.5 cm and greater than 0.5 cm in length.

Field measurements

Seedlings were removed after five weeks in cold storage and outplanted on June 3, 1997 to three forested sites at the University of Alberta Woodbend Field Station, Devon, Alberta. Seedling height, new shoot growth and RCD were measured on September 26, 1997, after approximately 16 1/2 weeks of growth. Needle length and needle density were also measured on the previous year's growth and on new field growth (n=15).

Statistical analysis

The experiment was a randomized block design, with four treatments and three blocks per treatment. ANOVAs were used to determine treatment effects and a Tukey's Studentized Range test (SAS statistical analysis software) was used to establish significant differences between treatment means. The reported data are presented as means ± standard error.

Results

Growth

Significant temperature regime effects ($p<0.001$) on seedling height were not evident until week 11, after the seedlings had entered their exponential growth phase (Figure 3.1a). At that time, the T1/2 and the T1/3 seedlings had significantly reduced height compared to the control seedlings, but there were no differences in height between the T1/4 and the control seedlings. Over the following 14 weeks, the general trend indicated that a reduction in height was proportional to the frequency of chilling exposure. By week 27, when growth had ceased and the seedlings were hardened off, the control seedlings were significantly ($p<0.001$) taller than those from the other three chilling treatments. There were no significant differences in height between the T1/4 and the T1/3 seedlings; however, the T1/2 seedlings were significantly shorter than seedlings from the remaining treatments.

Initially, RCD of control seedlings was larger ($P=0.041$) than that of the T1/2 or the T1/3 seedlings (Figure 2.1b). However, by week 15 there were no significant treatment effects on RCD, and this trend continued throughout the rest of the growth period. Apparent difference in RCD after week 13 were due to blocking rather than treatment effects.

Drought resistance

Initial shoot water potential measurements showed no significant treatment differences, with all seedlings having water potentials between -0.6 and -0.7 MPa (Figure 3.1). In response to drought, seedlings from all treatments showed a decline in water potential. However, nine days after the drought was initiated, shoot water potentials in the T1/2 seedlings were significantly ($p=0.03$) higher compared with those from the control treatment (Figure 3.2). Once the seedlings were re-watered, recovery from drought indicated a significant treatment effect ($p=0.0006$), with the shoot water potentials from all chilling treated seedlings recovering faster than the control seedlings (Figure 3.2).

Frost tolerance

TC seedlings showed signs of frost damage to needle tissues between approximately -5° and -7°C. T1/4, T1/3 and T1/2 seedlings showed damage between -7° and -9°C, with the T1/2 seedlings maintaining a higher percentage of green tissues at -7°C (Figure 3.3 a-f). These results are qualitative, based on the "browning test" commonly used in tree nurseries. Quantitative results from the electrolyte leakage test were not included as total electrolytes were not expressed from the shoot tissues by the method used in this experiment.

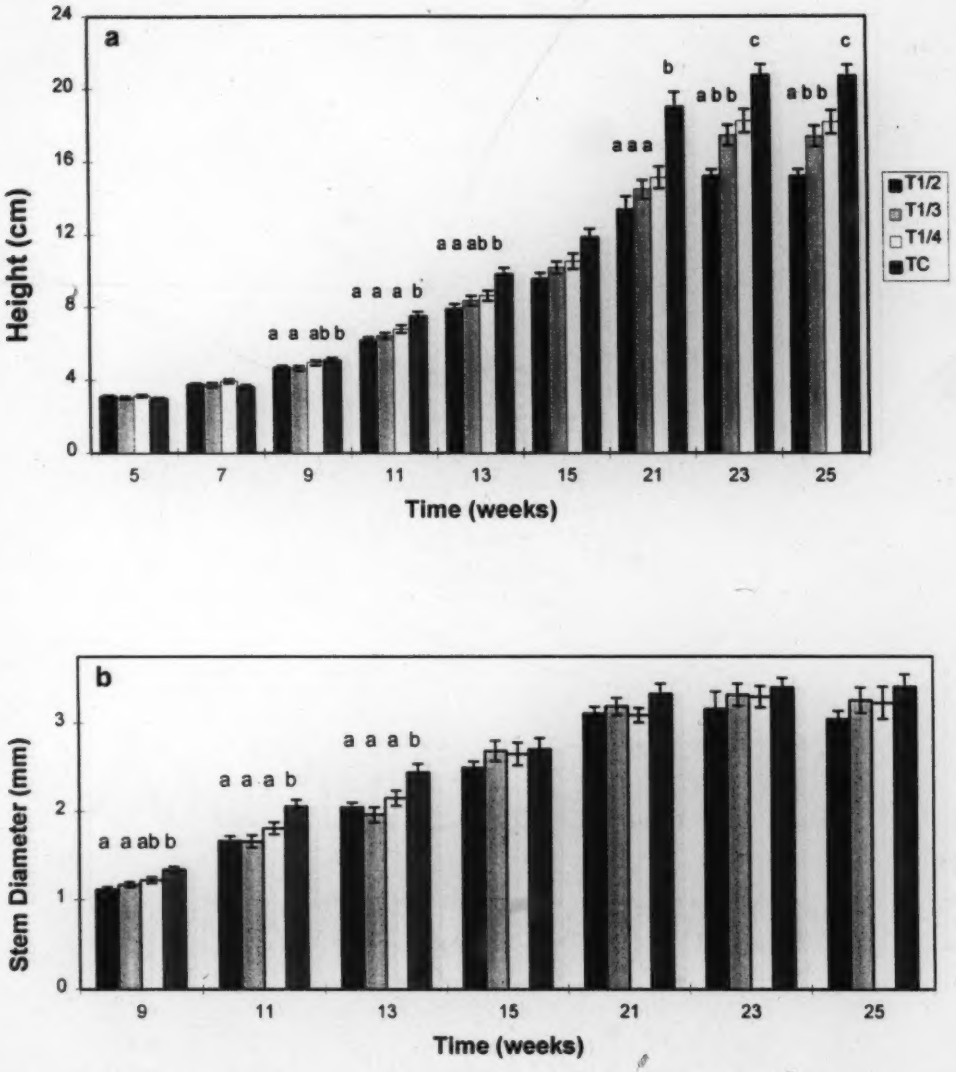


Figure 3.1. Shoot height (A) and RCD (B) of white spruce seedlings subjected to four temperature regimes. Means (± SE) are reported; n=45 seedlings per treatment. Means with the same letter on each sampling date are not statistically different at the 0.05 level, based on a Tukey's test.

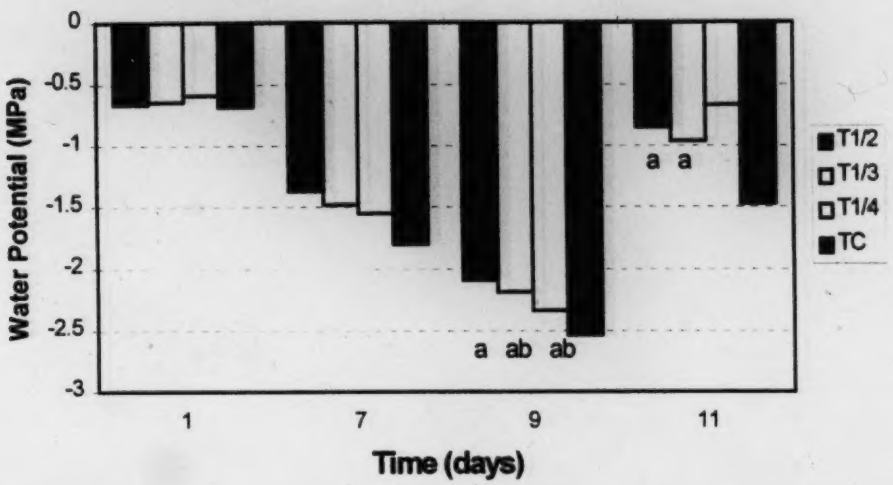
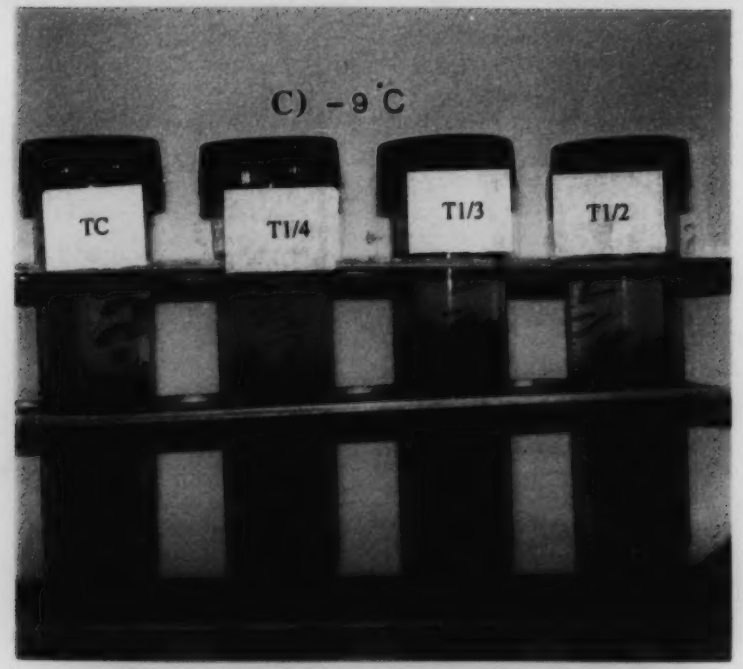


Figure 3.2 Shoot water potentials for seedlings subjected to a water stress for nine days. Seedlings were re-watered on day nine. Means (± SE) are reported; n=9 seedlings per treatment. Means with the same letter on each sampling date are not statistically different at the 0.05 level based on a Tukey's test.

Figure 3.2. Visual results of the frost tolerance test performed on the temperature treated seedlings. Green tissues indicate frost hardy seedlings, while brown tissues indicate frost damage(intolerance). A) -5°C, B) -7°C, D) -9°C, and e) -11°C.


C) -9°C
TC
T1/4
T1/3
T1/2

D) -11°C
TC
T1/4
T1/3
T1/2

Root growth capacity

After 21 days at 10°C there were significant treatment differences in the mean number of new roots per seedling in both the 0-0.5 cm (p=0.042), and over 0.5 cm (p=0.019) size classes. Seedlings from the T1/2 and the T1/3 treatments had the fewest roots from both size classes compared to the control seedlings, but there were no significant differences between the T1/4 and the control treatment seedlings in both cases (Figure 3.4).

Field experiment

After 16.5 weeks of growth in the field the T1/2 treatment seedlings showed the greatest increase in height (58%), compared to 37.8% in the control seedlings (Table 3.2). There were no significant treatment effects on either needle length or the number of needles produced before or after outplanting (Table 3.2). T1/2 seedlings had the smallest RCD before planting, followed by the T1/3 and T1/4 seedlings (Table 3.3). However, after field growth there were no significant differences in seedling RCD between the treatments. The treatments with the greater chilling exposure had the greatest percent increases in RCD over the control seedlings (Table 3.3). Seedling survival averaged over the three field sites (Table 3.3) ranged from 71.9% (T1/2 seedlings) to 80.8% (T1/3 seedlings).

Table 3.2. Seedling height, needle morphology and density for seedlings from the four temperature treatments before and after outplanting (n=30). Treatment means with the same letter within a column are not statistically different.

	Total Height (cm)			*Needle Length (cm)*		*Needle Density*	
	Initial	Final	Increase	Pre-planting	Post-planting	Pre-planting	Post-planting
T1/2	17.0 a	27.0 a	58.8%	2.01 a	1.35 a	23.0 a	17.6 a
T1/3	18.0 b	25.7 a	42.8%	1.97 a	1.32 a	22.4 a	21.8 a
T1/4	19.3 c	24.7 a	28.0%	2.03 a	1.38 a	24.2 a	21.0 a
TC	20.1 c	27.8 a	37.8%	1.89 a	1.13 a	20.6 a	20.6 a

Table 3.3. Root collar diameter (RCD) and percent survival for seedlings grown under one of four temperature treatments. Means with the same letter within a column are not statistically different.

	RCD (mm)			*Survival (%)*
	Initial	Final	% Increase	
T1/2	3.04 a	4.86 a	59.9	71.9
T1/3	3.16 b	4.85 a	53.5	80.8
T1/4	3.21 b	4.79 a	49.2	79.7
TC	3.33 c	4.94 a	48.3	78.3

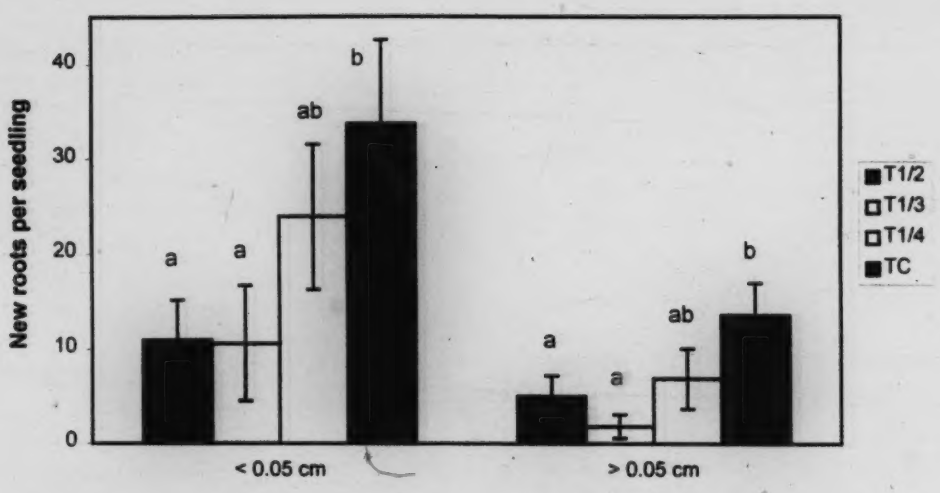


Figure 3.4. Root growth capacity of seedlings grown under one of four temperature treatments, then re-flushed under 10°C. Means (± SE) are reported, n=30 seedlings per treatment. Means with the same letter within each size class are not statistically different at the 0.05 level based on a Tukey's test.

Discussion

Exposing seedlings to 5°C for 24 hours every three to five days did not induce seedling bud set during the early stages of seedling development. Bud set in white spruce is usually induced by decreased photoperiod (Macey and Arnott 1986). However, when normal growing conditions are coupled with other stresses such as low temperatures, decreased nitrogen availability or increased moisture stress, buds can be induced to set (Macey and Arnott 1986; Landis *et al.* 1989, 1992).

The decrease in photosynthetic rates combined with reduced nutrient uptake at low temperatures could have contributed to the decrease in seedling size observed in the present experiment. Even relatively infrequent chilling exposure (the T1/4 treatment) caused a significant decrease in final seedling height compared to the control seedlings, although these effects were not apparent until the rapid growth phase. Low temperatures have been previously been shown to decrease rates of photosynthesis, respiration, transpiration and root hydraulic conductivity (Brix 1971; Wang 1982; Lopushinsky and Kaufmann 1984; Landis *et al.*1992). In this experiment, increasing the frequency of chilling exposure from every five days (the T1/4 treatment) to every four days (the T1/3 treatment) did not significantly affect final seedling height. This suggests that lowering greenhouse temperatures more frequently (every four days compared to every five) could lead to lower greenhouse heating costs without further affecting height differences. In the present study, seedlings from all four treatments remained within the industry-recommended range for white spruce seedlings (Wood 1995).

During the early growth phase, control seedlings had similar shoot lengths, but significantly greater stem diameters, compared with seedlings from the chilling treatments. This suggests that during initial seedling growth, periodic low temperature exposure influenced these tissues differently. Further anatomical studies are needed to determine exactly how and which tissues were influenced by the treatments during the various stages of seedling development. Seedling RCD values during the first few weeks of seedling growth appeared to be above the recommended nursery standards (Wood 1995). Since RCD is measured in millimeters, significant differences in stem diameter can easily occur, depending on the exact location at which the measurements were taken (Landis et al 1994). Other reasons for the high RCD values could be due to differences in how the measurements were made. In this study, the stem tissues were not completely woody during the early measurements, therefore diameter was recorded with the calipers gently touching the sides of the stem to avoid damaging soft tissues. Thompson (1992) recommends compressing the bark when making measurements. Once the seedlings were hardened off, the stems were woody and could not be easily compressed. Final seedling stem diameters from all treatments were within the recommended limits (Wood 1995).

In the present study, shoot water potentials were used as an indicator of internal moisture stress (Buxton *et al.* 1985). Results from the drought experiment suggested that frequent, periodic exposure to chilling temperatures during seedling growth may produce seedlings more resistant to a subsequent, severe drought stress. Lamhamedi *et al.* (1997) examined the effects of shoot height on water potentials from wet to dry watering regimes in black spruce, and found that in all cases, taller seedlings had an increased transpirational area which allowed for greater water vapor loss either through the stomata or across the cuticle. Van den Driessche (1991) also found that reduced seedling size, smaller height:diameter and shoot:root ratios were all associated with increased drought resistance in several species of conifers. However, for the current study, both morphological and physiological adaptations that developed in response to chilling could influence seedling drought stress resistance. Upon re-watering, there were significant treatment differences in seedling recovery. Shoot water potentials of chilled seedlings recovered faster, and closer to pre-stress levels than the control seedlings. It is known that ABA accumulates in chilling-acclimated plants and in some cases increased their root hydraulic conductivity (Markhart 1984), in addition to decreasing stomatal conductivity (Roberts and Dumbroff 1986). The faster recovery rate of the chilled seedlings could be due to increased hydraulic conductivity or decreased transpiration rates, however, seedling ABA levels were not measured in the present study.

Qualitative results from the frost tolerance test on unhardened seedlings suggested that increasing the frequency of chilling exposure during seedling growth will prevent frost damage at lower temperatures. However, this general conclusion was based on the visual results of a browning test, not on the quantifiable results from the electrolyte leakage method. Initial results were encouraging, and further study of periodic low temperature effects on the frost tolerance of unhardened seedlings are strongly recommended. Specifically, it would be important to determine at what developmental stage the treatment effects become apparent, and to repeat the frost tolerance measurements, but increase the resolution of the testing temperature from 2°C to 1°C.

Root growth capacity is used as a standard test in industry to determine stock quality (Ritchie 1985). Testing the new root growth of seedlings at optimal temperatures (root growth potential) has previously been criticized, as ideal growing conditions are rarely found on harvested sites during early seedling establishment (Grossnickle and Major 1994; Folk and Grossnickle 1997). Therefore, in the present study, root growth capacity was measured at 10°C. Frequent chilling exposure reduced the subsequent production of new roots at 10°C. However, since root biomass, density, length or morphology were not measured before the test, it is difficult to quantify the relative production of the new roots between seedlings from the different treatments. In addition, field measurements did not show that chilling treated seedlings maintained reduced seedling heights. Therefore it is questioned if reduced root growth in chilling treated seedlings is a significant disadvantage.

Results from the field study indicate that the initial reduction in seedling height, due to chilling exposure, did not restrict subsequent height growth. In fact, the most severely

chilling treated seedlings (T1/2) increased their height by over 58%. van den Driessche (1991) found similar results for Douglas-fir seedlings treated with low nursery temperatures. The study determined that low temperatures contributed to the production of well-formed terminal buds, with higher nursery temperatures resulting in fewer well-formed buds, which subsequently lowered the growth in the following year. However, van den Driessche (1991) also reported that low temperatures and increased drought stress in the nursery led to a decreased number of well-formed buds in white spruce seedlings, and therefore decreased height growth the following year.

In the present study, seedling field survival for all treatments was similar, but relatively low for all treatments. There were no significant treatment effects on needle length, either on previous year's needles, or after field growth. Conifer needle length has been found to decrease when acclimated to low temperatures or when grown under water stress (Thompson 1985; Alberdi and Corcuera 1991). Therefore, it appears that the periodic warm exposures allowed the seedlings enough time to recover from the temporary chilling so that they morphologically adapted to the warm environment as opposed to the chilling one. This suggests that incorporating periodic low temperatures into a nursery growing regime should not significantly affect the needle morphology, either after the initial growth, or after outplanting into the field.

The distance between needles can be influenced by tree density within the container, the light availability, or water stress (Thompson 1985). Rapid shoot expansion and competition for light availability results in reduced needle density. In the present study, there were no treatment differences in needle density, but there were differences in seedling height, suggesting that periodic chilling exposure affected similarly the shoot elongation and needle production. Again, this suggests that seedlings responded to chilling exposure by temporarily slowing shoot elongation, and increasing it again once placed under warm conditions. Therefore, the seedlings were adapting their shoot morphology to the warm, rather than the chilling conditions. Pre and post-planting differences in seedling RCD also suggest that the initial treatment effects on stem diameter were not long-lasting, since the differences were not evident after the first outplanting season. Percent increases in the stem diameter were proportional to the degree of initial chilling exposure, indicating that seedlings placed under the chilling exposure treatments could respond well to stressed environmental conditions.

Conclusions

Periodically reducing the greenhouse temperature is one alternative to the currently recommended growing regimes. In the present study, increasing the frequency of periodic chilling exposure from every five to every three days reduced seedling height, but this effect did not persist after the first season of field growth. There were no significant chilling effects on seedling field survival either. Needle length and density were not significantly affected by the chilling treatments. Chilling exposure increased the drought resistance and drought stress recovery of the seedlings, but these effects were only evident during or after a severe drought. Increases in frost tolerance of unhardened seedlings may occur; However further investigation into this area is needed. Further studies into the physiological effects of chilling on root production, root hydraulic conductivity and the mechanisms of drought resistance and frost tolerance are also recommended.

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Chapter IV: Synthesis

Tree nursery growers have historically relied on seedling morphological measurements to estimate the quality of a planting stock. Seedlings that are too tall may not have a well developed root system, and are therefore culled individually, or as a lot. Conversely, small seedlings are also removed, the belief being that slow growth in the nursery would reflect slow growth on the planting site. However, as greater understanding of seedling growth and physiology advanced over the years, new tests were developed to determine seedlot quality; predictive tests incorporating both morphological and physiological measurements to estimate seedling outplanting potential (Sutton 1979; Chavase 1980; Jaramillo 1980; Duryea 1985; Glerum 1988; Puttonen 1989; Simpson 1990; Grossnickle *et al.* 1991). However, there are two factors that tree growers often overlook when using these tests to assess the quality of a planting stock. The first is that predictive tests are often carried out under ideal or non-stressed conditions, which do not reflect how the seedlings will respond under realistic field conditions (Grossnickel *et al.* 1991a, 1991b; Folk and Grossnickle 1997; Mattsson 1997). The second is that nursery cultural practices will heavily influence the final quality of the seedling stock (Van den Driessche 1991a, 1991b; Folk and Grossnickle 1997; Mattsson 1997). Despite the advancements and studies that have shown these factors to be of value to tree growers, many nurseries have not incorporated them into their growing practices. Further, although it has been established that coordinating seedling stock types with future outplanting sites increases the reforestation success (Grossnickle *et al.* 1988; Proctor 1996), little research has been conducted as to how nursery growing practices could be modified to increase the overall quality of a planting stock.

The general purpose of this study was to test alternative growing regimes for tree nurseries which could produce seedlings that are better adapted to specific outplanting conditions, and to reduce nursery greenhouse heating costs without negatively affecting the quality of the planting stock. Since the experimental growing regimes were fundamentally different than conventional growing methods, comparison of treatment effects had to be made within seedlings from their respective experiment, as opposed to those from the nursery.

One of the most prominent results from both the humidity and the temperature experiments was that incorporating stress into growing practices resulted in decreased seedling shoot height. However, current nursery standards these seedlings would still be within an acceptable height range. In addition, both the low humidity and frequent chilling-exposed seedlings showed higher water potential levels during a severe water stress compared to the control or low stress treatments. This suggests that these growing regimes decreased the water stress on the seedlings. When comparing the height results to the current standards recommended by industry, it is apparent that tree nurseries are favoring their growing practices towards producing taller seedlings, regardless if there are increased beneficial physiological or morphological conditioning associated with shorter

seedlings. That is, the seedlings produced under the current growing regimes may not necessarily be the "best" seedlings for all outplanting conditions. When examining the growth of the low humidity and frequent chilling-exposed seedlings, after flushing under non-stressed or field conditions, it became apparent that treatment effects on height were not persistent. This suggests that the nursery-induced stress treatments confer a general increase in stock quality that could be beneficial to seedlings in the early establishment phase after outplanting. Further investigations into these alternative growing regimes are warranted. Current growing practices must be assessed to determine if a single, generic growing method is acceptable for producing stock that is to be planted on either low-stress or high-stress planting sites.

Caution is suggested when recommending these exact growing regimes to container-tree growers. Factors that were taken into account when designing these experiments, and that must be considered if these regimes are to be considered by a nursery, are: tree species; the location (latitude) of the tree nursery; and the level of environmental control over greenhouse growing conditions. White spruce seedlings were ideal for this experiment because they initiate terminal bud set in response to a change in photoperiod. In comparison, other high elevations or interior species such as Englemann spruce, Scotch pine or mountain hemlock may set a bud in response to other stresses (possibly including exposure to low temperature or low RH), regardless of the photoperiod. Northern latitude tree nurseries that would introduce stressful growing regimes into their current practices must ensure that their photoperiod is adequate, as even one incident of altered photoperiod may result in bud set, especially if the seed source represents a sensitive species or ecotype. Finally, incorporation of the experimental treatments may only be possible if the greenhouse used for growing the containerized seedlings is under adequate temperature control. Since one of the current experiments involved low RH, it must be emphasized that humidity is a temperature dependent parameter. Modifying a greenhouse to remove excess water vapor (thus decreased humidity) will be less effective if the temperature of the greenhouse is under poor control, as warmer temperatures will increase the water vapor saturation level of the air. Increasing the temperature control over the greenhouse may be more effective in controlling the atmospheric humidity level than pumping out the water vapor from the growing rooms. In addition, temperature regulation of a greenhouse should be under control of a thermostat if implementing the low-temperature treatments. Larger greenhouses with incorrectly placed temperature sensors may result in some of the seedlings freezing, as re-heating of the air may take too long when heaters do not start up soon enough, or if the thermostats are not registering the correct temperature for seedlings placed close to the outer perimeter of the greenhouse.

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